

Chlamydial isolates from Reiter's syndrome*

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Evidence has accumulated concerning the association of micro-organisms of the genus *Chlamydia* with Reiter's syndrome (RS). Isolations have been reported from the conjunctiva, genital tract, and synovial fluid or tissue, and antichlamydial complement-fixing (CF) antibodies have been demonstrated in the sera of patients (Schachter, Barnes, Jones, Engleman, and Meyer, 1966; Schachter, 1967; Kinsella, Norton, and Ziff, 1968; Ostler, Dawson, Schachter, and Engleman, 1971). However, the incidence of positive CF tests was reported (Kinsella and others, 1968) to be no greater than that seen in patients attending a venereal disease clinic who had no evidence of RS. Although most cases of RS are related to venereal exposure (Ford, 1968), non-gonococcal urethritis (NGU) and conjunctivitis associated with *Chlamydia* may follow venereal contact, and occur without additional signs that identify RS (Dunlop, Freedman, Garland, Harper, Jones, Race, Du Toit, and Treharne, 1967; Schachter, Rose, and Meyer, 1967). Furthermore, CF tests for chlamydial infection are regularly performed with a group antigen, and may be positive after infection with any type of chlamydial agent. Other serological evidence has been reported (Phillips and Christian, 1970) which implicates measles, paramyxovirus I, or a virus antigenically related to these, as possibly associated with RS. Norton (1969) reviewed the question of RS-*Chlamydia* association and analysed

the difficulties of interpreting available evidence. His conclusion is still valid, that an aetiological relationship between RS and *Chlamydia* has not been established.

Improved methods of isolation of *Chlamydia* from the conjunctiva and genital tract (Gordon, Harper, Quan, Treharne, Dwyer, and Garland, 1969; Philip, Hill, Greaves, Gordon, Quan, Gerloff, and Thomas, 1971) and of antigenic characterization (Wang, 1971) of strains of *C. trachomatis* are now available and have been used in the present study. This report describes the isolation in cell culture of chlamydial strains from the eye and urethra of a patient with RS, as well as cultural, pathological, and serological studies on the isolates. The isolation, establishment of strains, and the observations on morphology and drug susceptibility were performed in the Naval Medical Research Institute. Pathogenicity tests and serotyping were accomplished in the Rocky Mountain Laboratory.

Case report

A 36-year-old man developed severe persistent bilateral conjunctivitis in August, 1967. Standard bacterial cultures of his conjunctiva were negative. A sulphathiazole ophthalmic solution and an antibiotic-steroid combination ophthalmic ointment were unsuccessful in controlling the conjunctivitis, and a month later the patient developed a symptom complex of progressive malaise, diarrhoea, dysuria, and arthralgia. Urine culture was negative at this time. Except for the malaise, the symptoms disappeared within 2 weeks without treatment.

In November, 1967, the patient was seen by one of us (T.S.) for the first time because of his persistent conjunctivitis, malaise, and acute symptoms of an upper respiratory tract infection. He also complained of recent onset of bad breath associated with soreness of the gums.

EXAMINATION

He was afebrile, but there was marked erythema of and exudate from both palpebral conjunctivae which showed a cobblestone appearance. Tender preauricular adenopathy was present. Slight redness of the gums was noted,

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but there were no specific mucocutaneous lesions. The joints were normal.

INITIAL LABORATORY TESTS

Haematocrit 45 per cent.; haemoglobin 14.4 g./100ml, blood leucocyte count 8,200/cu. mm. (48 per cent. neutrophils, 51 per cent. lymphocytes, 1 per cent. eosinophils); erythrocyte sedimentation rate 8 mm./1st hr.

Urine acid, with specific gravity 1.016; tests for sugar and albumin negative. Microscopic examination of the sediment of a mid-stream clean-catch urine specimen showed 15-20 leucocytes and 2-3 erythrocytes per high-power field.

Antinuclear factor not detected. Heterophil test for infectious mononucleosis negative. Uric acid elevated to 8.4 mg. per cent.

Following blood tests normal: glucose, calcium, phosphorus, electrolytes, blood urea nitrogen, creatinine, cholesterol, SGOT, total protein, alkaline phosphatase.

Chest x ray and electrocardiogram within normal limits.

Routine bacterial cultures of urine and conjunctiva negative.

Specimens for chlamydial isolation were taken at this time.

TREATMENT AND PROGRESS

Hourly treatment with 15 per cent. sulphacetamide ophthalmic solution resulted in marked improvement in the patient's conjunctivitis over the next month. In December, 1967, he developed acute arthritis involving the knees, ankles, elbows, and shoulders. There was no involvement of the small joints. Associated with the arthritis was an exacerbation of the conjunctivitis, diarrhoea, and dysuria. A 14-day course of tetracycline, 250 mg. four times a day was followed by improvement in all symptoms. The joint symptoms slowly improved over the next 5 months, and there was no clinical or x-ray evidence of joint destruction or deformity. Although the conjunctivitis cleared, the patient experienced a crusting of both eyelids upon awakening every morning and swelling of the right upper tarsus.

In November, 1968, he developed pneumonia which rapidly responded to penicillin therapy. There was no exacerbation of his previous symptoms at that time.

ISOLATION OF *Chlamydia trachomatis*

The method employed for isolation and passage of chlamydiae in cell culture has been described in detail elsewhere (Gordon and others, 1969). It consists essentially of centrifugation of specimens and culture harvests onto monolayers of McCoy cells, previously irradiated to promote formation of giant cells (Gordon, Dressler, Quan, McQuilkin, and Thomas, 1972). At 48 hrs. fixed monolayers are stained with an iodine solution or by Giemsa stain, and examined for intracellular inclusions. In the case of *C. trachomatis*, the inclusions are strongly stained by iodine because of their glycogen content. At 72 hrs other cultures are harvested and passed, and the series is continued.

The specimens were obtained in November, 1967, by passing cotton swabs vigorously over the surfaces of the palpebral conjunctivae, gums, and distal third of the urethra. The swabs were then agitated in 1.0 ml. of a storage medium, SP (0.2 M sucrose and 0.102 M phosphate buffer, pH 7.2), to suspend absorbed material, and the suspension was stored at -65°C . For inoculation of cell cultures some weeks later, the thawed specimen was diluted 1:5 with cell culture medium (Eagle's minimal essential medium with added amino acids, glutamine, 10 per cent. horse serum, 100 $\mu\text{g./ml.}$ vancomycin, and 50 $\mu\text{g./ml.}$ streptomycin) and cultured as described above.

Cultures of the urethral specimen were positive at the first passage; 65 characteristic inclusions were counted in the 120 microscopic fields examined in the two coverslips, indicating a figure of 528 inclusion-forming units (IFU) per ml. in the suspended specimen. The cultures of the conjunctival specimen were reported as questionable (two inclusions of uncertain character), and those of the gums as negative. At the second passage the urethral isolate, now designated as NMR-22, gave an increased inclusion count, and the conjunctival specimen also proved to be positive, showing 24 IFU/ml. of first-passage harvest; this isolate is called NMR-21.* The second passage of the buccal specimen was negative, as was the third passage, after which the series was discontinued. Extreme care was taken, as described in an earlier study (Gordon and others, 1969), to avoid extraneous or cross contamination during inoculations and passages. This consists of the use of separate sterile cabinets and equipment for each operation with disinfection after each use. Eventual serotyping of the isolates revealed that no strains of this type had been in our laboratory previously or at the time of isolation.

Continued passage of the two isolates in cell cultures soon resulted in high infectivity titres, although a number of passages had to be made in the presence of vancomycin and streptomycin to eliminate a persistent bacterial contamination. Both strains were also passaged in the yolk sac of embryonated eggs. Storage of yolk sac or cell culture harvest was in SP at -65°C . Unfortunately, no serum specimens from the patient were available for tests with chlamydial antigens.

UNSUCCESSFUL ISOLATION ATTEMPTS

In the past few years we have made numerous other similar but unsuccessful attempts to isolate *Chlamydia* from cases, or suspected cases, of RS. We have examined 54 specimens from fifteen patients, as follows: synovial fluid 12; synovial tissue 6; urethra 9; conjunctiva 12; skin lesions 5; rectal biopsy 7; prostatic fluid 2; blood clot 1. For these we are indebted to Dr. Robert Sylvester, Naval Hospital, Bethesda, and to Drs. M. S. Artenstein, R. L. Keepers, L. H. Shainkler, and S. Zane, Walter Reed Army Medical Center, Washington, D.C.

*The complete designations of these isolates, following the recommendations of Gear, Gordon, Jones, and Bell (1963), are TRIC/I/USA/NMR-21/0 and TRIC/I/USA/NMR-22/G.

Observations on isolates

The two species now recognized in the genus *Chlamydia*, *trachomatis* and *psittaci* (Page, 1968), are differentiated primarily on the basis of morphology of inclusions (Gordon and Quan, 1965) and susceptibility to sulphonamides and d-cycloserine (Moulder, 1964), differences that correlate with separation by serological examination (Terzin, Vedros, and Johnston, 1964; Vedros, 1967). The significance of differentiation on these bases was confirmed by the finding that representative strains of the two species are completely unrelated when tested by reassociation of isolated deoxyribonucleic acids (Kingsbury and Weiss, 1968). Within the species, *C. trachomatis*, a number of serotypes are now established (Wang and Grayston, 1971).

NMR-21 and NMR-22 both produce typical inclusions of *C. trachomatis* in infected cells stained by Giemsa, or iodine, and the latter reveals the presence of glycogen, characteristic of *C. trachomatis*. Suppression of growth in cell culture occurred in the presence of either 20 µg. sulphadiazine or 30 µg. d-cycloserine/ml. culture medium—results identical to those obtained with other typical strains of *C. trachomatis* (Gordon and Quan, 1972). There concentrations do not significantly inhibit a representative strain of *C. psittaci* (meningopneumonitis).

Strains of *C. trachomatis* may vary in pathogenicity for mice and for embryonated eggs. The two isolates reported here possessed low pathogenicity for the egg (low egg infectious dose/lethal dose ratios), similar to that previously found (Philip and others, 1971) with isolates from simple genital tract infections, in contrast to the high pathogenicity (ratios near unity) obtained with isolates from lymphogranuloma venereum (LGV) tested in the same manner. NMR-21 and NMR-22 possessed little or no pathogenicity for mice, again in contrast to LGV isolates. The identification of these strains as *C. trachomatis*, possessing little pathogenicity for mouse

and yolk sac, is in contrast to the report (Schachter 1967) that preliminary results with some other RS isolates revealed properties characteristic of *C. psittaci*.

Antigens of the two isolates were tested with a series of mouse antisera against established serotypes within *C. trachomatis*, by the fluorescent antibody technique described by Wang (1971). Antisera prepared against NMR-21 and NMR-22 were also tested with antigens of the established immunotypes. Both isolates proved to be similar or identical to three strains (DC049, DC044, DC055) previously reported (Philip and others, 1971) and which were derived, respectively, from a male urethra (NGU), and the cervixes of two women with essentially subclinical infections. These, in turn, were found by Wang, Grayston, and Gale (1973) to fall within an immunotype now designated as I. NMR-21 and NMR-22 were also examined by Wang who stated (personal communication) that he found that they also belonged to Type I. The positive results of the immunotyping performed in the present study are presented in the Table. All other tests, in which both antisera and antigens of serotypes A, B, C, D, E, F, LGV-II, and LGV-III were employed, were essentially negative with respect to NMR-21 and NMR-22. In a very few instances minimal titres were detected, eight to sixteen-fold lower than the homologous titre.

Comment

Although strong circumstantial evidence implicates *Chlamydia* as an aetiological agent of RS, based especially on its isolation from joints, ambiguity will continue until confirmatory reports appear and additional studies on isolates are made. It is important to know whether chlamydial isolates from RS possess distinguishing characteristics, antigenic or otherwise. The present report indicates that two isolates, from the conjunctiva and urethra of a patient with RS,

TABLE Serotyping of the two isolates, NMR-21 and NMR-22, by microimmunofluorescence

Mouse antisera prepared against	Antigens					
	DC044	DC049	DC055	NMR-21	NMR-22	(Serotypes A, B, C, D, E, F, LGV-II, LGV-III)
DC044	512	512	512	1,024	512	
DC049		256		256	256	
DC055	1,024	1,024	1,024	1,024	512	
NMR-21	256	256	128	1,024	512	0-16
NMR-22	128	256	128	512	256	0-16
(Strains representing serotypes A, B, C, D, E, F, LGV-II, LGV-III)				0-16	0-16	*

Figures indicate the reciprocal of the highest dilution of sera giving detectable fluorescence.

*Homologous titres were 128 to 1,024.

were identified as *C. trachomatis*, were similar to each other, resembled in pathogenicity other non-LGV genital isolates, and belonged to Wang's serotype I which mainly includes strains from the male urethra and from the cervix.

Isolations of *Chlamydia* from RS specimens have been infrequent. Our total of successful isolations in one patient of sixteen (2 of 57 specimens positive) may be compared with those of one report in which no isolations were made from fourteen patients (Ford and McCandlish, 1971), and another in which isolations were successful in six patients out of a series of 23 (14 of 133 specimens positive) (Ostler and others, 1971). The last group of investigators also employed direct staining of specimens by Giemsa and fluorescent antibody. Their total figure for demonstration of *Chlamydia* by any of the three methods was twelve positive in the total of 23 (see also Dawson, Schachter, Ostler, Gilbert, Smith, and Engleman, 1970).

Factors influencing the incidence of positive tests no doubt include the stage of the disease at which specimens are taken and the medication which has been given. Some of our specimens were obtained during apparently acute stages, others when one or more features of the disease were quiescent. We attempted to exclude specimens from any patient recently treated with antibacterial drugs, but at least one such patient was included in the series.

It is possible that RS may be initiated by any one of several disease agents, although there is strong correlation with venereal exposure (Ford, 1968). *Chlamydia* may be present in affected parts at some stage of many venereally acquired cases of RS, as suggested by increased antibody levels, but such stages may be of short duration, or may occur before the diagnosis is established and attempts to culture the organism are made. It seems less likely that RS is associated with chlamydial strains that are especially difficult to recover by our methods. Our success with the patient described above may have been due to the time at which we obtained the specimens. Although arthralgia had appeared and allowed at least a tentative diagnosis of RS, the specimens were taken approximately one month before the onset of acute arthritis.

Summary

Isolates of *Chlamydia* were obtained from the eye and urethra of a patient with Reiter's syndrome by inoculation of cell cultures. Specific identification as *C. trachomatis* was accomplished by noting the morphology of the intracellular inclusions and by

determining sensitivity to sulphadiazine and d-cycloserine. Their low level of pathogenicity for mice and embryonated eggs emphasizes the similarity of these strains to others derived from uncomplicated infections of the genital tract and eye. Serotyping placed the new isolates in Wang's group I.

Similar isolation attempts with 54 specimens from fifteen other patients were unsuccessful. Procurement of the specimens at an early stage, before acute arthritis had appeared, may have been significant in the single success achieved.

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References

- DAWSON, C. R., SCHACHTER, J., OSTLER, H. B., GILBERT, R. M., SMITH, D. E., and ENGLEMAN, E. P. (1970) *Arch. Ophthalm. (Chicago)*, **83**, 300
- DUNLOP, E. M. C., FREEDMAN, A., GARLAND, J. A., HARPER, I. A., JONES, B. R., RACE, J. W., DU TOIT, M. S., and TREHARNE, J. D. (1967) *Amer. J. Ophthalm.*, **63**, 1073
- FORD, D. K. (1968) *Canad. med. Ass. J.*, **99**, 900
- and MCCANDLISH, L. (1971) *Brit. J. vener. Dis.*, **47**, 196
- GEAR, J. H. S., GORDON, F. B., JONES, B. R., and BELL, S. D., Jr. (1963) *Nature (Lond.)*, **197**, 26
- GORDON, F. B., DRESSLER, H. R., QUAN, A. L., MCQUILKIN, W. T., and THOMAS, J. I. (1972) *Appl. Microbiol.*, **23**, 123
- , HARPER, I. A., QUAN, A. L., TREHARNE, J. D., DWYER, R. St. C., and GARLAND, J. A. (1969) *J. infect. Dis.*, **120**, 451
- and QUAN, A. L. (1965) *Ibid.*, **115**, 186
- — (1972) *Antimicrob. Agents Chemother.*, **2**, 242
- KINGSBURY, D. T., and WEISS, E. (1968) *J. Bact.*, **96**, 1421
- KINSELLA, T. D., NORTON, W. L., and ZIFF, M. (1968) *Ann. rheum. Dis.*, **27**, 241
- MOULDER, J. W. (1964) "The Psittacosis Group as Bacteria." Wiley, New York.
- NORTON, W. L. (1969) *Ann. Rev. Med.*, **20**, 351
- OSTLER, H. B., DAWSON, C. R., SCHACHTER, J., and ENGLEMAN, E. P. (1971) *Amer. J. Ophthalm.*, **71**, 986
- PAGE, L. A. (1968) *Int. J. syst. Bacteriol.*, **18**, 51
- PHILIP, R. N., HILL, D. A., GREAVES, A. B., GORDON, — and QUAN, A. L. (1965) *Ibid.*, **115**, 186
- — (1972) *Antimicrob. Agents Chemother.*, **2**, 242
- PHILLIPS, P. E., and CHRISTIAN, C. L. (1970) *Science*, **168**, 982
- SCHACHTER, J. (1967) *Amer. J. Ophthalm.*, **63**, 1082
- , BARNES, M. G., JONES, J. P., ENGLEMAN, E. P., and MEYER, K. F. (1966) *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 283
- , ROSE, L., and MEYER, K. F. (1967) *Amer. J. Epidemiol.*, **85**, 445
- TERZIN, A. L., VEDROS, N. A., and JOHNSTON, J. B. (1964) *J. Hyg. (Lond.)*, **62**, 185

- VEDROS, N. A. (1967) *J. Immunol.*, **99**, 1183
 WANG, S-P. (1971) In "Trachoma and Related Disorders",
 ed. R. L. Nichols, p. 273. Int. Congr. Ser. No. 223.
 Excerpta Medica, Amsterdam
 — and GRAYSTON, J. T. (1971) *Idem*, p. 305
 —, —, and GALE, J. L. (1973) *J. Immunol.*, **110**, 873

Isolements de *Chlamydia* dans le syndrome de Reiter

SOMMAIRE

Des *Chlamydia* furent isolés de l'oeil et de l'urètre, par inoculation de cultures cellulaires, chez un malade

atteint de syndrome de Reiter. *C. trachomatis* fut identifié d'après la morphologie des inclusions intra-cellulaires et la détermination de la sensibilité à la sulfadiazine et à la d-cycloserine. Le faible taux de pathogénicité pour la souris et pour l'oeuf embryonné souligne que ces souches sont semblables à celles provenant d'infections non compliquées des voies génitales et de l'oeil. L'étude sérotypiques a situé les nouveaux isolements dans le groupe Wang I.

Des essais d'isolement similaires furent effectués sur 54 échantillons provenant de 15 autres malades et échouèrent. Dans le seul cas qui se montra positif, il est possible que le fait que le prélèvement ait été positif à la phase aigüe, avant que l'arthrite aigüe soit apparue, puisse avoir eu un rôle déterminant dans cette unique réussite.